



## Three new species of *Ophryotrocha* (Annelida: Dorvilleidae) from a whale-fall in the North-East Atlantic

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### Abstract

Three new *Ophryotrocha* species are described from sites with high levels of organic carbon flux including a whale-fall at 125 m depth off the Swedish coast and sediment sampled at 104 m depth beneath a fish farm in a Norwegian fjord. Phylogenetic analyses based on the nuclear gene H3 and the mitochondrial genes COI and 16S using MrBayes and Maximum Likelihood analyses show that *Ophryotrocha eutrophila* sp. nov. is a close relative to *Ophryotrocha puerilis*, while *Ophryotrocha craigsmiti* sp. nov. falls together with *Palpiphitime lobifera*, and *Ophryotrocha scutellus* sp. nov. occur within the 'hartmanni' clade. The genus *Ophryotrocha* is in our study monophyletic only if the genera *Iphitime* and *Palpiphitime* are included. Two representatives of *Ophryotrocha* previously described from anthropogenically-enriched sediments are here reported for the first time in very high abundance from a naturally occurring habitat. We suggest that whale falls are important habitats for the evolution of ecosystem services such as the degradation of complex organic compounds.

**Key words:** Polychaeta, organic enrichment, phylogeny, chemosynthetic ecosystem

### Introduction

Whale carcasses that sink to the sea floor constitute a substantial addition of nutrients that can be utilised by benthic organisms as food (Smith & Baco, 2003). After the soft tissue is consumed by mobile scavengers, the bones can still sustain a diverse fauna for many years, where some species consume the complex organic carbons within the bones (e.g. *Osedax* Rouse et al., 2004) or use the diverse microbial communities that form bacterial mats at the surface of the bones. New species from several different annelid genera have been recently described from whale-fall habitats, e.g. five species of the siboglinid genus *Osedax* from the Pacific (Rouse et al., 2004; Fujikura et al., 2006; Rouse et al., 2008) and Atlantic Ocean (Glover et al., 2005), two species of the chrysopetalid genus *Vigtorniella* (Kiseleva, 1992) from the Pacific (Dahlgren et al., 2004) and Atlantic Ocean (Wiklund et al., 2009) and the new hesionid genus *Vrijenhoekia* Pleijel et al., 2008 described from a whale-fall off California. In this study we describe three new species of *Ophryotrocha* Claparède & Meczников, 1869 found on an experimentally implanted whale-fall at 125 m depth in the north east North Atlantic (Dahlgren et al., 2006), and report on dense populations of two additional *Ophryotrocha* species previously described from nutrient rich substrates of anthropogenic origin (e.g. cellulose fiber discards from a pulp mill; Åkesson, 1973).

Worms from the dorvilleid genus *Ophryotrocha* are often found in organically enriched or even heavily polluted areas, such as in harbours and beneath marine aquaculture plants. The genus has a complicated systematic history. It was erected with *Ophryotrocha puerilis* Claparède & Meczников, 1869 as type species although the authors described a mixture of characters from *O. puerilis* and the species that was later to be

formally described as *Ophryotrocha labronica* La Greca & Bacci, 1962. Recently, both these species have been redescribed and neotypes have been assigned to them (Paxton & Åkesson, 2007). These authors also point at morphological and behavioural characters to help separate the subspecies *O. puerilis siberti* (McIntosh, 1885) from *O. puerilis puerilis* Claparède & Mecznikov, 1869, e.g. sclerotization of mandibles and reproductive differences. The two subspecies are included in our study as two different taxa to estimate the molecular differences between them and test the validity of the current subspecies status of the two forms.

*Ophryotrocha* has traditionally been placed within the polychaete family Dorvilleidae Chamberlin, 1919. Orensanz (1990) moved *Ophryotrocha* from Dorvilleidae to Iphitimidae Fauchald, 1970. He also erected three new genera within Iphitimidae and moved some *Ophryotrocha* species to two of the new genera (Orensanz, 1990). However, Hilbig (1995), and Eibye-Jacobsen & Kristensen (1994) both refuted the moving of *Ophryotrocha* to Iphitimidae, based partly on finds of new species that possessed characters from both family definitions. More recent, higher-level molecular analyses including dorvilleid taxa also suggest major problems in delineating the taxon (e.g. Struck et al., 2006). Here we follow Eibye-Jacobsen & Kristensen (1994) and consider *Ophryotrocha* and the other genera from Iphitimidae as belonging to Dorvilleidae, which consists of 33 genera as defined by Eibye-Jacobsen & Kristensen (1994).

The first phylogenetic study based partly on genetic data done on *Ophryotrocha* combined morphological characters with investigations on chromosome counts of 20 *Ophryotrocha* and three outgroup taxa (Pleijel & Eide, 1996). It was later followed by Dahlgren et al. (2001) who used 16S as the molecular marker in their study containing 18 *Ophryotrocha* species and five outgroup taxa. Heggoy et al. (2007) undertook a molecular study of *Ophryotrocha* with both 16S and cytochrome c oxidase I (COI), and they also included one species from the genus *Iphitime* Marenzeller, 1902 to investigate its position. In their study, *Iphitime* fell within *Ophryotrocha* (Heggoy et al., 2007), as has been suggested previously, e.g. by Hoisaeter & Samuelsen (2006). In this study, phylogenetic relationships of the new dorvilleid species are investigated using one nuclear (H3) and two mitochondrial (16S and COI) markers in analyses containing 18 species from the genus *Ophryotrocha*, two *Iphitime* species and seven outgroup taxa. Furthermore we have included two species from *Palpiphitime* Orensanz, 1990, *Palpiphitime lobifera* (Oug, 1978), which Orensanz moved from *Ophryotrocha* and used as type species when he erected *Palpiphitime*, and the newly described species *Palpiphitime lipovskya* Paxton, 2009.

## Material and methods

### (a) Sample collection and morphological analysis

Polychaetes were sampled using remotely operated vehicle (ROV), from a minke whale carcass at 125 m depth that was implanted in October 2003 in Kosterfjord off the Swedish west coast, 58° 53.1' N; 11° 06.4' E (Dahlgren et al., 2006). Bones from the whale-fall were collected using the manipulator arm of the ROV and placed in a sealed plastic box on the seafloor. On retrieval, polychaetes were either sampled from the bones directly or from sieving the water from the box that the bones were enclosed in on a 90 µm sieve. Bones from the carcass were kept in experimental aquaria with sand-filtered running sea water at 7°C, and some individuals were collected from the whale bones in these tanks. Animals were relaxed in 7% magnesium chloride in distilled water, photographed alive, and preserved for scanning electron microscopy (SEM), DNA analyses and standard morphology. Specimens for SEM were fixed in 1% osmium tetroxide in filtered seawater for 15 minutes, rinsed in distilled water and stored in 70% ethanol, critical point dried, gold-coated and imaged using a Hitachi S-4300. Specimens for DNA sequencing were preserved in 95% ethanol and stored in -20°C, and specimens for standard morphology were fixed in 10% formalin in seawater for one hour, and transferred to 70% ethanol. The holotypes and vouchers for the sequenced species in this study are deposited at the Swedish Museum of Natural History (SMNH) in Stockholm, Sweden, voucher numbers in Table 1. Paratypes are deposited at the Natural History Museum (NHM) in London, UK. All animals not deposited as type material or specimen vouchers are in the first author's collection.

**TABLE 1.** Taxa, collection sites, voucher numbers and NCBI GenBank accession numbers.

Terminal taxa	Locality	Voucher	H3	16S	COI
<i>Dorvillea albomaculata</i> Åkesson & Rice, 1992	GenBank		---	AF380115	EF464550
<i>Dorvillea erucaeformis</i> (Malmgren, 1865)	GenBank		---	AY838827	AY838868
<i>Dorvillea rubrovittata</i> (Grube, 1855)	Istra, Croatia	SMNH106071	GQ415490	GQ415457	---
<i>Dorvillea similis</i> (Crossland, 1924)	GenBank		---	DQ317915	DQ317857
<i>Eunice pennata</i> (O.F. Müller, 1776)	GenBank		DQ779731	AF321418	AY838870
<i>Iphitime hartmanae</i> Kirkegaard, 1977	Koster Area, Sweden	SMNH106072	GQ415491	GQ415458	GQ415472
<i>Iphitime paguri</i> Fage & Legendre, 1934	GenBank		---	---	EF464549
<i>Ophryotrocha alborana</i> nom. nud.	Ceuta, Spain	SMNH106073	GQ415492	AF321422	GQ415473
<i>O. craigsmithi</i> sp. nov.	Sweden and Norway	SMNH106087	GQ415493	GQ415459	GQ415474
<i>O. eutrophila</i> sp. nov.	Koster Area, Sweden	SMNH106088	GQ415494	GQ415460	GQ415475
<i>O. geryoncola</i> (Esmark, 1874)	Väderöarna, Sweden	SMNH106074	GQ415495	GQ415461	GQ415476
<i>O. globopalpata</i> Blake & Hilbig, 1990	Juan de Fuca Ridge	SMNH106075	GQ415496	GQ415462	GQ415477
<i>O. gracilis</i> Huth, 1933	Sylt, Germany	SMNH106076	GQ415497	AF321424	EF464545
<i>O. hartmanni</i> Huth, 1933	GenBank		---	AF321419	EF464546
<i>O. japonica</i> nom. nud.	Tsushima, Japan	SMNH106077	GQ415498	GQ415463	GQ415478
<i>O. labronica</i> La Greca & Bacci, 1962	Hurghada, Egypt	SMNH106078	GQ415499	AF321429	GQ415479
<i>O. lipovskyae</i> (Paxton, 2009)	British Columbia, Canada	SMNH106238	---	---	GQ415480
<i>O. lobifera</i> Oug, 1978	Sweden and Norway	SMNH106079	GQ415500	GQ415464	GQ415481
<i>O. longidentata</i> Josefson, 1975	Koster Area, Sweden	SMNH106080	GQ415501	GQ415471	GQ415482
<i>O. maculata</i> Åkesson, 1973	Koster Area, Sweden	SMNH106081	---	GQ415465	GQ415483
<i>O. notoglandulata</i> Pfannenstiel, 1972	GenBank		---	AF321431	EF464542
<i>O. permanni</i> nom. nud.	Xiamen, China	SMNH106082	GQ415502	AF321432	GQ415484
<i>O. puerilis puerilis</i> Claparède & Mecznirow, 1869	Malaga, Spain	SMNH106083	GQ415503	GQ415466	GQ415485
<i>O. puerilis siberti</i> (McIntosh, 1885)	Malaga, Spain	SMNH106084	GQ415504	GQ415467	GQ415486
<i>O. robusta</i> nom. nud.	GenBank		---	AF321433	EF464547
<i>O. rubra</i> nom. nud.	Ceuta, Spain	SMNH106085	GQ415505	GQ415468	GQ415487
<i>O. scutellus</i> sp. nov.	Sweden and Norway	SMNH106086	GQ415506	GQ415469	GQ415488
<i>Parougia eliasoni</i> (Oug, 1978)	Koster Area, Sweden	SMNH106089	GQ415507	GQ415470	GQ415489
<i>Protodorvillea kefersteini</i> (McIntosh, 1869)	GenBank		DQ779759	DQ779634	AY598738

**(b) DNA analysis**

In the molecular analyses, 29 taxa were included (Table 1), 18 from *Ophryotrocha*, ten taxa from other genera within Dorvilleidae, and one outgroup taxon. The outgroup taxon was chosen from another eunicid family, *Eunice pennata*, since Eunicidae in some phylogenetic studies has been suggested to be closely related to Dorvilleidae (e.g. Struck et al., 2006). Five of the *Ophryotrocha* species in this study are not yet formally described. They are presented under the names they will be described (Paxton & Åkesson, in prep.), followed by nom. nud. Some sequences in the study were obtained from NCBI GenBank (Table 1). Extraction of DNA was done with DNAeasy Tissue Kit (Qiagen) following the protocol supplied by the manufacturer. About 400 bp of 16S, 330 bp of H3, and 600 bp of cytochrome c oxidase subunit I (COI) were amplified. PCR mixtures contained ddH<sub>2</sub>O, 1 µl of each primer (10µM), 2 µl template DNA and puReTaq Ready-To-Go PCR Beads (GE Healthcare) in a mixture of total 25 µl. The temperature profile was as follows: 96°C/240s –(94°C/30s–48°C/30s–72°C/60s)\*45cycles–72°C/480s. PCR products were purified with the E.Z.N.A. Cycle-Pure Kit (Omega Bio-tek). Sequencing was performed by the MacroGen Sequencing System in Korea, on an ABI 3730XL DNA Analyser (Applied Biosystems), using primers listed in Table 2.

Overlapping sequence fragments were merged into consensus sequences using Geneious (Drummond et al., 2007) and aligned using Clustal X 2.0 (Thompson et al., 1997) with default settings (15/6.66 as gap/gap length penalties). All regions that could not be unambiguously aligned were excluded. Alignments are available at TreeBase, <http://www.treebase.org>, accession number SN2462. The datasets were tested for incongruence using the Shimodaira-Hasegawa (SH) test in PAUP\*, with RELL (resampling estimated log-likelihood) 1000 bootstrap replicates. The trees within the 95% confidence interval from the separate analyses made in MrBayes were used in the test. The SH test showed that the partitions were not incongruent and the three datasets were combined. The computer program PAUP\* 4.0b10 (Swofford, 2002) was used for the parsimony (PA), and Maximum Likelihood (ML) analyses, with heuristic search and TBR (tree bisection and reconnection) branch swapping. Clade support was assessed using non-parametric bootstrap with 5000 replicates and ten random additions in PA, and with 100 replicates in ML. For the ML analysis, the three molecular datasets were combined and run in ModelTest (Posada, 1998), which suggested GTR+I+G as the best model. Bayesian phylogenetic analyses (BA) were conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Analyses were run three times with the combined dataset with four chains for 2,000,000 generations. 400,000 generations were discarded as burn-in. The results from each dataset were compared, and when values approached similar mean values for all parameters they were considered to have converged. The evolutionary models used for the molecular data in BA were obtained by running the separate datasets in MrModelTest (Nylander, 2004), and for 16S the optional model was GTR+I+G. For COI and H3, the data was partitioned into codon positions. For COI, position 1 and 3 followed GTR+G, while GTR+I+G was used for position 2, while for H3 position 1 followed GTR+I, position 2 followed JC+I and GTR+G was used for position 3. In the combined Bayesian analysis, the data was partitioned into the three parts (16S, H3, COI), the evolutionary models mentioned above were applied to each partition and corresponding codon position respectively, and the parameters used for the partitions were unlinked.

**TABLE 2.** PCR and sequencing primers.

Primer	Sequence 5'-3'	References
16SarL	CGCCTGTTTATCAAAAACAT	Palumbi (1996)
16SbrH	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)
H3F	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (2000)
H3R	ATATCCTTRGGCATRATRGTGAC	Colgan et al. (2000)
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
COI-E	TATACTTCTGGGTGTCCGAAGAATCA	Bely and Wray (2004)

## Results

### (a) Systematics

#### **Dorvilleidae Chamberlin, 1919**

#### ***Ophryotrocha* Claparède and Mecznirow, 1869**

#### ***Ophryotrocha scutellus* sp. nov. (Figs 1A–D)**

Material examined: Northern North Atlantic, coastal Skagerrak, 58° 53.1' N; 11° 06.4' E, female with eggs, 6 mm long, 29 chaetigers, preserved in formaldehyde from experimental tank with bone material sampled from a minke whale carcass, which was implanted at 125 m depth, holotype (SMNH T-7816); same location, 2 specimens, preserved in formaldehyde, paratypes (NHM2009.25); same location, one specimen preserved in

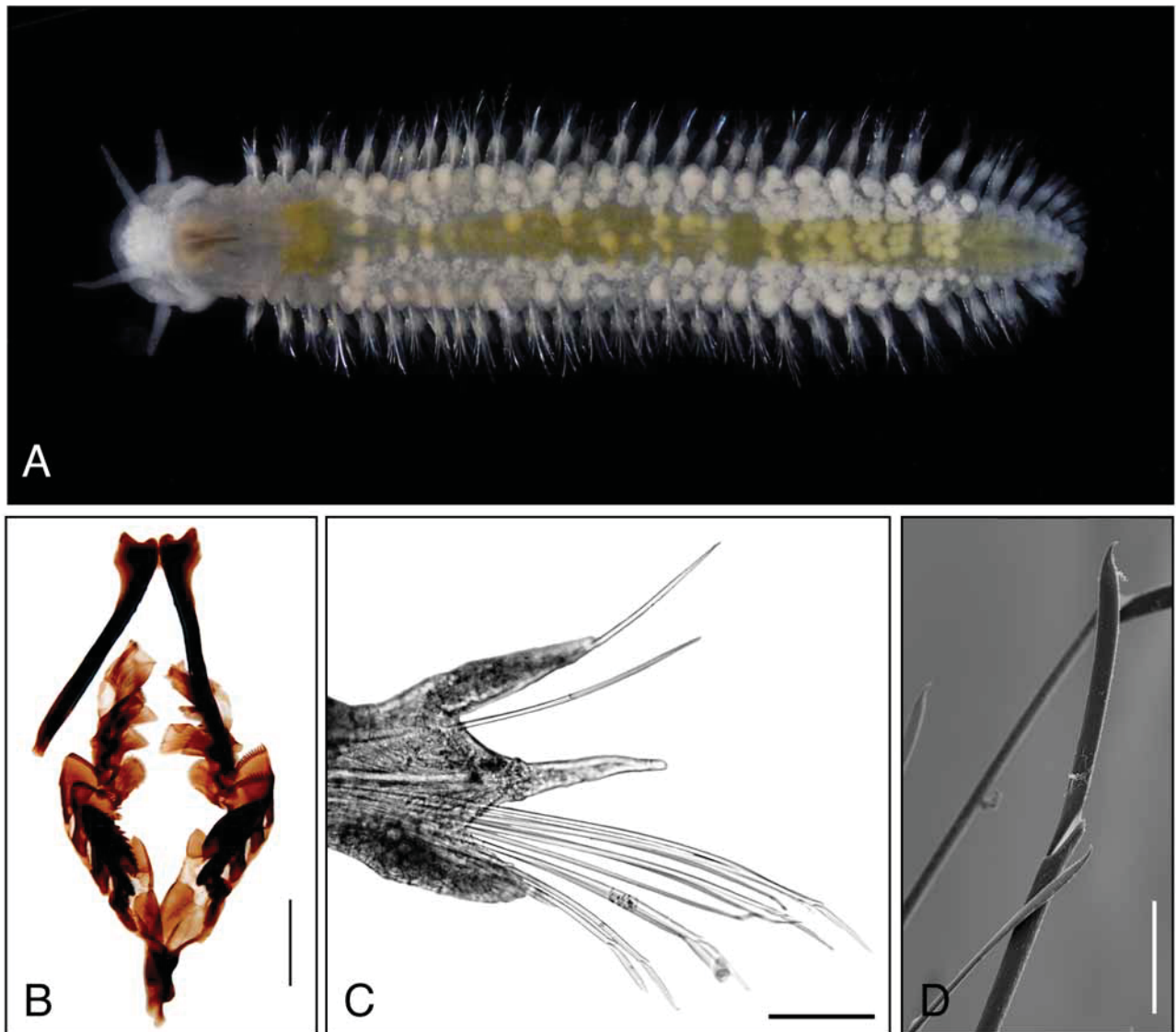


osmium for SEM, and several specimens preserved in ethanol for DNA extraction. Fishfarm in Mele, Hardangerfjord, 60°21.27'N; 6°20.89'E, 104 m depth, several specimens preserved in formalin.

Description: Body shape elongated, uniform width for majority of body length, tapering slightly at posterior end. Colour transparent, with white eggs visible in females. (Fig. 1A).

Prostomium round and dorso-ventrally flattened, disc-like. Eyes lacking. Long cirriform paired antennae inserted dorsally, reaching to first chaetiger, equally long palps cirriform inserted lateroventrally on prostomium. Jaws of P-type, mandibles rod-like without any serration. Maxillae with seven pairs of free denticles (Fig. 1B).

Two peristomial achaetous segments. Parapodia uniramous with long dorsal and ventral cirri and cirriform acicular lobe, supraacicular chaetae simple, subacicular chaetae compound with serrated blades (Figs 1C–D). Subacicular chaetal lobe with simple chaeta.



**FIGURE 1.** *Ophryotrocha scutellus* sp. nov., (A) live photo, (B) light micrograph of jaws, (C) light micrograph of parapodium, (D) SEM micrograph of chaetae. Scale bars in (B) and (C) are 100  $\mu$ m, in (D) 20  $\mu$ m.

Pygidium with terminal anus, two pygidial cirri as long as antennae and palps laterally and a short, nub-like unpaired appendage ventrally.

Distribution: Known from a minke whale carcass at 125 m depth (58°53.1'N; 11°06.4'E) in the Koster area in Sweden, and from sediment sampled at 104 m depth beneath a fish farm in Hardangerfjord (60°21.27'N; 6°20.89'E) in Norway.

Reproduction: Eggs present in females from chaetiger 5 and in all segments to posterior end of body. No data available on the presence of sperm.

Ecology: Live observation in aquarium experiments show adult specimens crawling on filamentous bacterial mats on the whale bones, and bacterial pellets are present in the worms guts, indicative of a bacterial diet.

Etymology: *Ophryotrocha scutellus* is named after its flattened disc-like head, scutella is the latin word for flat dish or saucer.

Remarks: *Ophryotrocha scutellus* has a rounded dorso-ventrally flattened head-form, shaped like a disc. Another *Ophryotrocha* that is reported to have flattened prostomium is *O. platykephale*, from which *O. scutellus* differs in jaw morphology, form of parapodia and absence of branchiae. Accession numbers for DNA sequences from *O. scutellus*, published on GenBank: GQ415469 (16S), GQ415488 (COI), GQ415506 (H3).

### ***Ophryotrocha craigsmithi* sp. nov. (Figs 2A–D)**

Material examined: Northern North Atlantic, coastal Skagerrak, 58° 53.1' N; 11° 06.4' E, female with eggs, 7 mm long, preserved in formaldehyde, from experimental aquaria containing bones sampled from a Minke whale carcass, which was implanted at 125 m depth, holotype (SMNH T-7817); same location, one specimen, not complete, preserved in formaldehyde, paratype (NHM2009.26), same location three specimens preserved in ethanol for DNA extraction. Fishfarm in Svåsand, Hardangerfjord, 84 and 150 m depth, ten specimens preserved in ethanol for DNA extraction.

Description: Colour pale red or transparent with red branchia-like structures on dorsal and ventral sides, the dorsal being very large and rounded in form, partly covering the dorsum (Fig. 2A). Body shape elongated, tapering slightly at posterior end.

Prostomium with digitiform paired antennae inserted dorsally. Palps papilliform with palpophores, inserted laterally on prostomium. No eyes. Jaws of P-type, mandibles L-shaped with serration anteriorly. Maxillae with 7 free denticles (Fig. 2B)

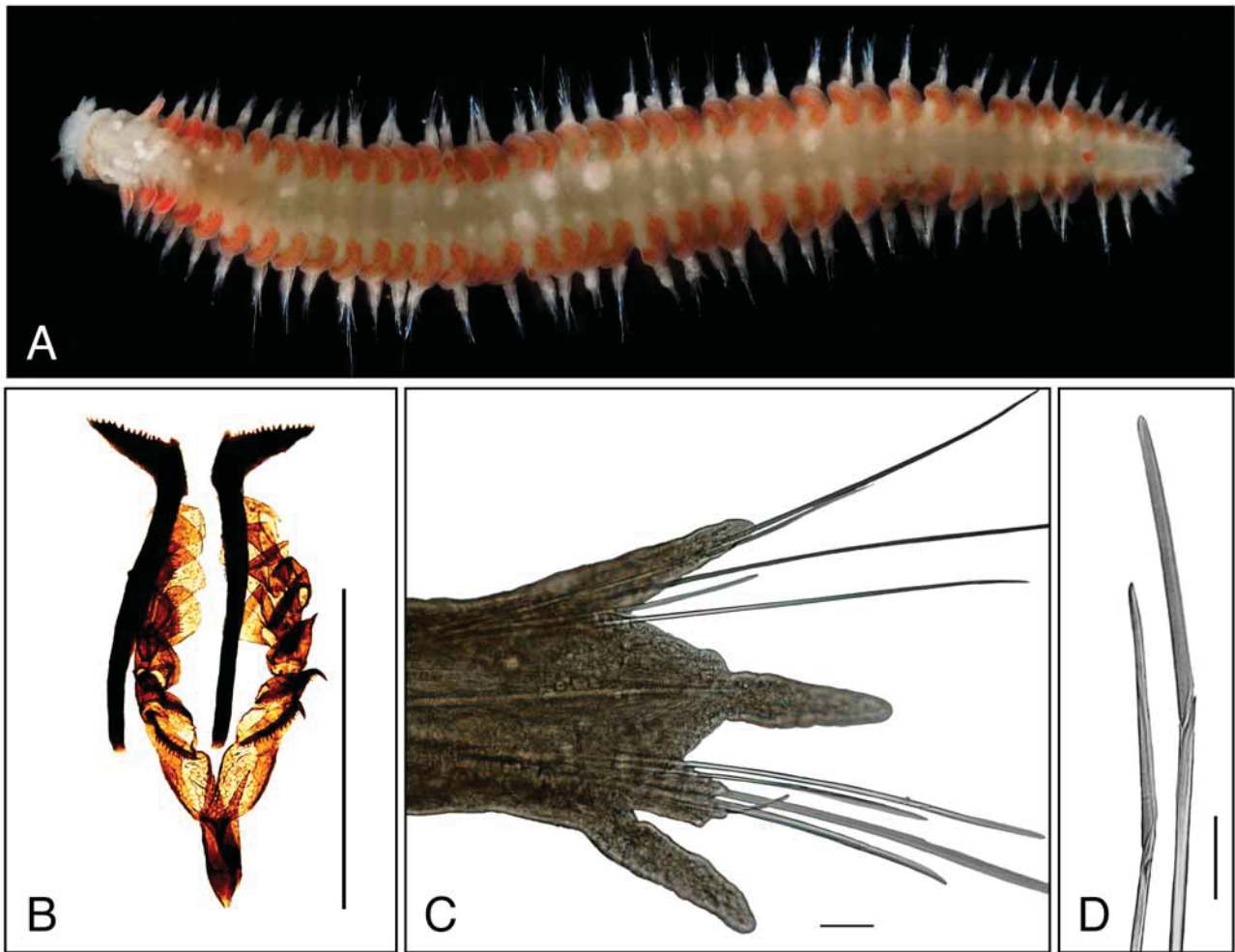
Two peristomial achaetous segments. Parapodia uniramous with dorsal and ventral cirri and cirriform acicular lobe, supraacicular chaetae simple, subacicular chaetae compound with serrated blades (Figs 2C–D). Subacicular setal lobe with simple chaetae.

Pygidium with terminal anus and two pygidial cirri, unpaired appendage absent.

Distribution: Known from the minke whale carcass at 125 m depth (58°53.1'N; 11°06.4'E) in the Koster area in Sweden, and from sediment sampled at 84 and 150 m depth beneath a fish farm in Hardangerfjord in Norway.

Etymology: *Ophryotrocha craigsmithi* is named after Professor Craig R. Smith in recognition of his encompassing work with whale fall habitats.

Remarks: This species is similar to *Palpiphitime lipovskya*, *O. platykephale* Blake, 1985, *O. wubaolingi* Miura, 1997 and *P. lobifera* in having branchial structures both dorsally and ventrally. It differs from *O. platykephale* in the form of prostomium and parapodia, from *O. wubaolingi* in the shape of the parapodia, and from *P. lobifera* in the form of the dorsal branchial structures, and in the absence of eyes. It seems to be most similar to the recently described *P. lipovskya* from sediment beneath fish farms in western Canada. *Palpiphitime lipovskya* is reported to have jaws of both P- and K-type. So far no specimens of *O. craigsmithi* have been found with K-type jaws, but it can not be ruled out that it does not possess them. *Ophryotrocha craigsmithi* differs from *P. lipovskya* genetically, and in the presence of a prominent ventral chaetal lobe with a protruding simple chaeta in *O. craigsmithi*. Accession numbers for DNA sequences from *O. craigsmithi*, published on GenBank: GQ415459 (16S), GQ415474 (COI), GQ415493 (H3).



**FIGURE 2.** *Ophryotrocha craigsmithi* sp. nov., (A) live photo, (B) light micrograph of jaws, (C) light micrograph of parapodium, (D) light micrograph of compound chaetae. Scale bar in (B) is 500 µm, in (C) 100 µm, in (D) 25 µm.

***Ophryotrocha eutrophila* sp. nov. (Figs 3A–F)**

Material examined: Northern North Atlantic, coastal Skagerrak, 58° 53.1' N; 11° 06.4' E, female with eggs, 8 mm long, 32 chaetigers, preserved in formaldehyde from experimental tank with bone material sampled from a minke whale carcass, which was implanted at 125 m dept, holotype (SMNH T-7818); same location, four specimens, two males and two females, preserved in formaldehyde, paratype (NHM2009.27); same location, seven specimens preserved in formaldehyde, two specimens preserved in osmium for SEM, and several specimens preserved in ethanol for DNA extraction.

Description: Colour transparent, females with eggs distinctly larger than males (Figs 3A, B). Body shape elongated, of generally uniform width, tapering slightly at posterior end.

Prostomium with digitiform paired antennae inserted dorsally. Palps papilliform, inserted laterally on prostomium. No eyes. Mandibles rodlike, with anterior dentition. K-type maxillae with smooth forceps and 7 pairs of free denticles (Fig. 3D). Maxillae of P-type with 7 free denticles (Fig. 3E).

Two peristomial achaetous segments, parapodia uniramous with short dorsal and ventral cirri (Fig. 3F), supraacicular simple chaetae with serration distally, subacicular chaetae compound, blades with serration (Fig. 3C), subacicular chaetal lobe with simple chaeta.

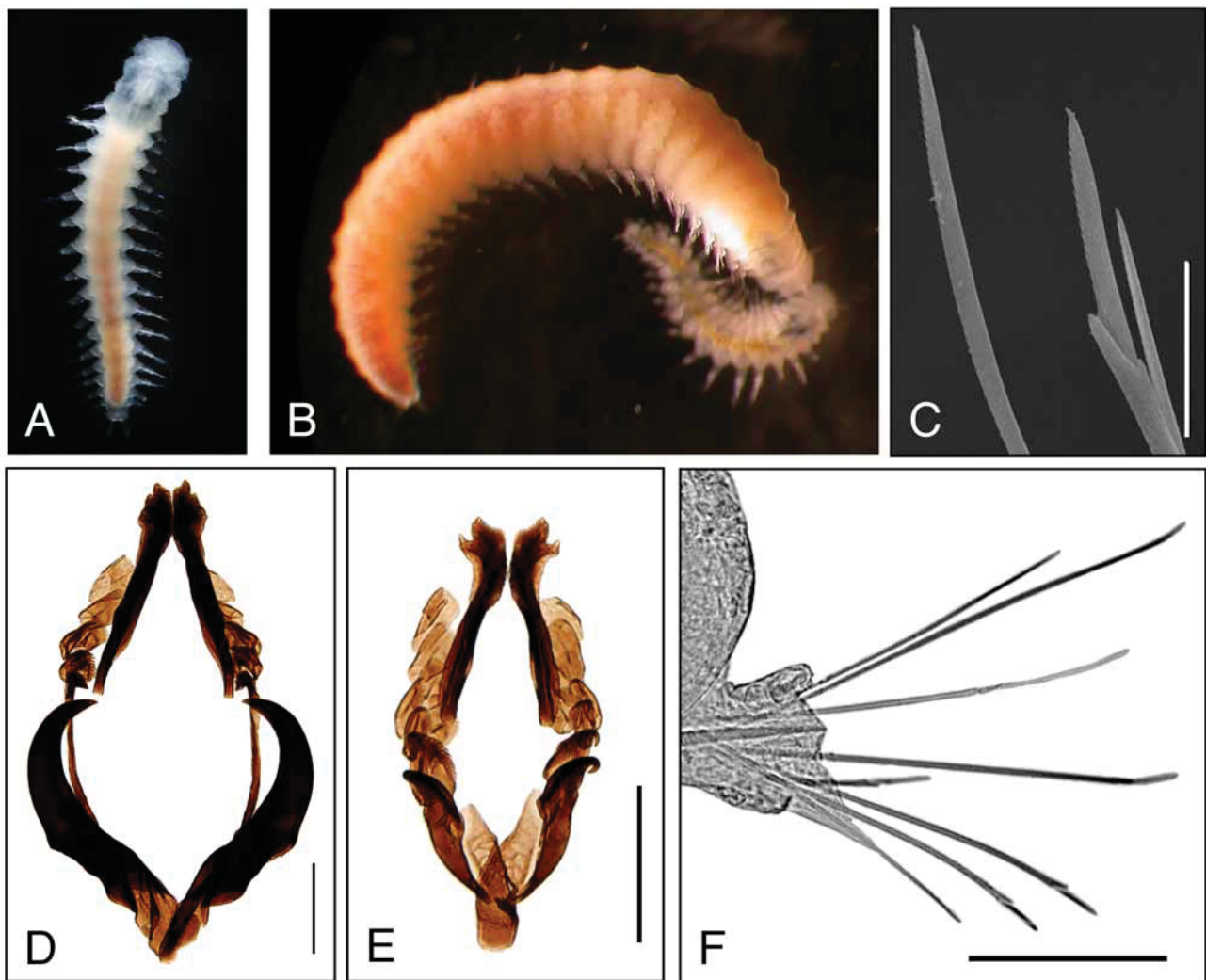
Pygidium with terminal anus, two pygidial cirri laterally inserted and an unpaired appendage ventrally placed.

Distribution: Known from an aquarium containing bones taken from a minke whale carcass at 125 m depth (58°53.1'N; 11°06.4'E) in the Koster area in Sweden.

Reproduction: Egg masses form a tube in which the female crawl, the tube loosely attached and not covered by a hard surface like in *O. labronica* (Paxton & Åkesson, 2007). No data on the distribution of eggs or sperm among the segments of the worms.

Etymology: *Ophryotrocha eutrophila* is named after its habitat choice, seemingly liking organically enriched environments (eutrophic=organically enriched, philus=like).

Remarks: This species resembles *O. puerilis* in jaw morphology. *Ophryotrocha eutrophila* is dimorphic, with males commonly smaller than females and possess K-type maxillae, similar to *O. puerilis*. *Ophryotrocha eutrophila* is genetically different from *O. puerilis* and differs in the absence of eyes and the presence of a well developed median pygidial stylus. *Ophryotrocha eutrophila* is also similar to *O. fabriæ* Paxton & Morineaux, 2009 described from a hydrothermal vent on the Mid-Atlantic Ridge. It differs from *O. fabriæ* in the form of the mandibles. Accession numbers for DNA sequences from *O. eutrophila*, published on GenBank: GQ415460 (16S), GQ415475 (COI), GQ415494 (H3).



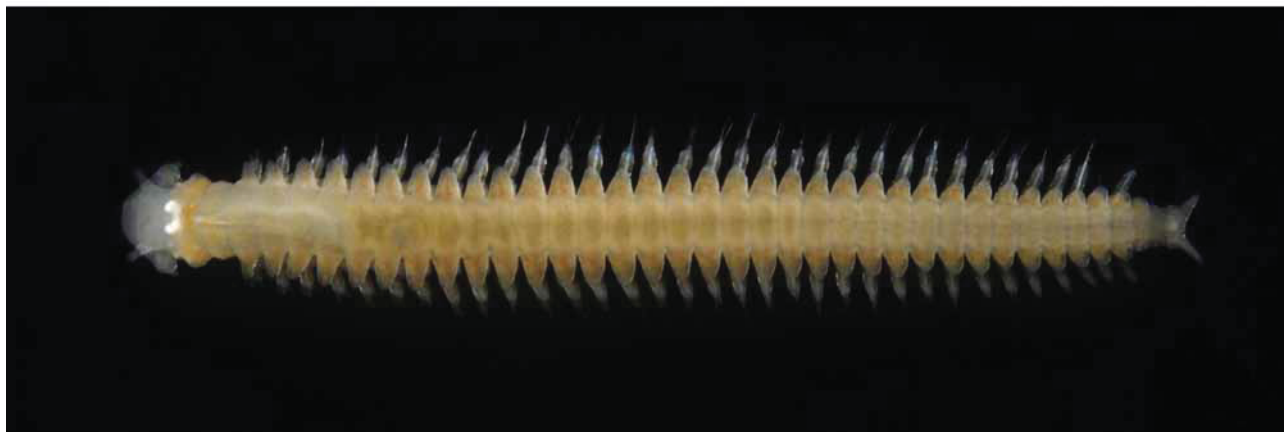
**FIGURE 3.** *Ophryotrocha eutrophila* sp. nov., (A) live photo of male specimen, (B) live photo of smaller male and larger female, (C) SEM micrograph of chaetae, (D) light micrograph of K-type jaws, (E) light micrograph of P-type jaws, (F) light micrograph of parapodium. Scale bar in (C) is 20  $\mu$ m and in (D), (E) and (F) 100  $\mu$ m.



***Palpiphitime lobifera* (Oug, 1978) (Fig. 4)**

Material examined: Northern North Atlantic, coastal Skagerrak, 58° 53.1' N; 11° 06.4' E. Specimens recovered from minke whale bones in experimental aquarium tanks.

Remarks: The most abundant dorvilleid on the whale bones is *Palpiphitime lobifera*. We here include a live photo of the species (Fig. 4) to show the presence of eyes, which were not reported in the original description (Oug 1978). The eyes are easily visible on live animals, but may be difficult to see in preserved specimens.



**FIGURE 4.** Live photo of *Palpiphitime lobifera* from whale-fall in Sweden.

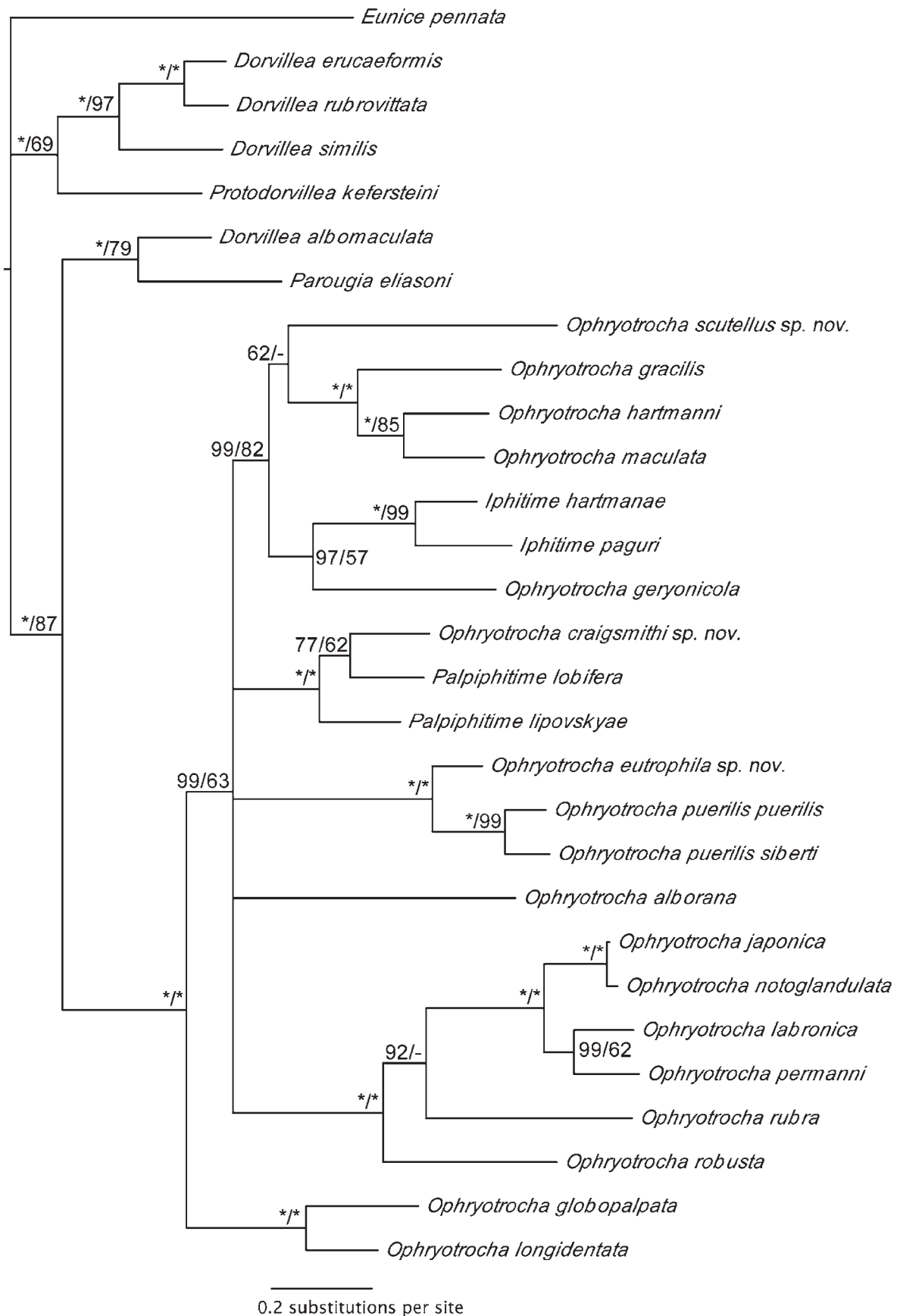
***Ophryotrocha maculata* Åkesson, 1973**

Material examined: Northern North Atlantic, coastal Skagerrak, 58° 53.1' N; 11° 06.4' E. Specimens recovered from minke whale bones in experimental aquarium tanks.

Remarks: As with *Palpiphitime lobifera* this species was abundant on the whale bones. Both species have been described from anthropogenically enriched sediments such as pulp mill discharge. The discovery of dense populations of these two species actively feeding on mat-forming filamentous bacteria at whale falls suggest that they are important species for ecosystem function in terms of the degradation of high molecular weight organic compounds.

**(b) Phylogenetic analyses**

The combined dataset consists of 1384 characters, 732 are variable, of which 643 are parsimony-informative. The three Bayesian analyses (BA) converged on similar log-likelihood values, mean values for all parameters, and clade probabilities. The 50%-majority rule consensus tree from the BA supports 23 nodes, of which 20 with clade credibilities >95% (Fig. 5). The ML 50%-majority rule consensus tree supports 23 nodes, all of which were also recovered by the BA, and 12 of these have bootstrap values above 95%. The bootstrap of the parsimony analysis (PA) provided >50% support for 16 nodes (not shown), all of which are present in the BA and ML. There are no topological incongruencies between the three analyses, and the differences between them relate solely to weaker or no support in ML and PA for some groups that were recovered in the BA. The *Ophryotrocha* clade including two *Iphitime* species has a strong support in all analyses (Fig. 5). *Dorvillea albomaculata* is sister to *Parougia eliasoni* instead of clustering with the other *Dorvillea* Parfitt, 1866, supporting the hypothesis that *D. albomaculata* belong to the genus *Parougia* Wolf, 1986 based on the absence of maxillary basal carriers (Paxton, pers comm).



**FIGURE 5.** Phylogenetic analyses of a combined dataset with three genes, majority rule consensus tree from the Bayesian analyses with posterior probability /bootstrap values from the analyses in MrBayes/Maximum Likelihood respectively, '\*' indicates support value of 100, '-' indicates that the node is not supported.

## Discussion

Although representatives of *Ophryotrocha* have been known for over a century, the increasing frequency in which apparently undescribed species of the genus is reported from the deep sea, hydrothermal vents, hydrocarbon seeps, and whale-falls suggest that the largest part of the *Ophryotrocha* species diversity is yet to be discovered. If this is indeed the case we have only sampled a fraction of the taxa for our analysis of phylogenetic relationship within the group. A small taxon sample would usually result in a less well resolved tree (e.g. Graybeal, 1998). We are therefore surprised by the high degree of resolution in our tree where 87 % of the nodes have posterior probability support values of 95 or higher.

*Ophryotrocha* has a complex systematic history, having been moved between families and also been split up into several genera (Orensanz, 1990). In this study, we have included in the analyses not only the species that Orensanz used as type species when erecting *Palpiphitime*, but also the genus *Iphitime*, for which some authors (e.g. Höisaeter & Samuelson, 2006; Heggoy et al., 2007) have argued belongs within *Ophryotrocha*. In our molecular analyses, both *Palpiphitime* and *Iphitime* are within the *Ophryotrocha* clade (Fig. 5). To maintain *Palpiphitime* and *Iphitime* as valid genera, *Ophryotrocha* would need to be divided into even more genera.

The resulting tree in this study (Fig. 5) agrees with a previous molecular phylogeny analyses of the *Ophryotrocha* which found two main clades within *Ophryotrocha*, the 'labronica' group with the gonochoristic species, and the 'hartmanni' group with hermaphrodites (Dahlgren et al., 2001). In our study, *Ophryotrocha* is divided into more clades. However, the 'labronica' group is still well-supported, and none of our new species are within that clade. Two of our new species, *O. scutellus* and *O. eutrophila*, occur in the 'hartmanni' group. *Ophryotrocha scutellus* falls out in a clade with *O. gracilis*, *O. hartmanni* and *O. maculata*, although the support for that clade is low (Fig. 5). The sister clade to them consists of the two *Iphitime* species and *O. geryonicola*, all of which are commensals on crustaceans. Within the *O. scutellus* - *O. geryonicola* clade, the span of divergence in morphological characters is quite large, with *O. gracilis* - *O. maculata* all fitting the original *Ophryotrocha* genus description. *Ophryotrocha scutellus* have long antennae and palps and a very flattened head, and *O. geryonicola* are commensals or parasitic in crustaceans and is one of the largest *Ophryotrocha*, reaching up to at least 110 mm for several hundreds of segments (Pfannenstiel et al., 1982). *Iphitime* was placed within its own genus and family due to its distinct morphological appearance with, for example, branchiae. The close relationship between the species living as commensals has been suggested by e.g. Gaston & Benner (1981), who suggested reinstating the genus *Eteonopsis* for *O. geryonicola* to separate it from the free-living species of *Ophryotrocha*. In our molecular study there is support for a close relationship between the two iphitimids and *O. geryonicola*, and the support for them all belonging within *Ophryotrocha* is strong, but an analysis including the type species *Iphitime doederleini* Marenzeller, 1902 would be needed to formally synonymize *Iphitime* with *Ophryotrocha*.

The third new species, *O. craigsmithi*, is sister to *Palpiphitime lobifera*. As mentioned before, Orensanz (1990) erected the new genus *Palpiphitime* using *P. lobifera* as type species, and justified it based on the species' biarticulated palps and dorsal and ventral lobes, which he considered to fall outside of the genus description for *Ophryotrocha*. A new species of *Palpiphitime*, *P. lipovskya*, was described recently (Paxton, 2009), and *O. craigsmithi* is morphologically very similar to this species, although they differ in that the latter species has a ventral chaetal lobe, and they also differ genetically. Hilbig and Blake (1991) disagreed with Orensanz' splitting of *Ophryotrocha* and extended the *Ophryotrocha* genus description to fit a wider heterogeneity in morphological characters, which would then include also *Palpiphitime*. Paxton (2009) extended Orensanz' definition of the genus *Palpiphitime* with jaw characters such as 2 transverse serrated ridges on P-forceps. However, based on our molecular analyses, *Palpiphitime* seems to belong within *Ophryotrocha* as it is delineated today. A revision of the *Ophryotrocha* genus is needed, but until that has been published we include the *Palpiphitime* species in *Ophryotrocha*. That is, for now we opt for *Palpiphitime lobifera* and *P. lipovskya* to be called *Ophryotrocha* and it is under that genus name the sequences have been submitted to GenBank.

The type species of the genus, *Ophryotrocha puerilis*, is in our molecular analyses sister to the new species *O. eutrophila*, which differs from *O. puerilis* morphologically in that it has no eyes, and that it has a well developed median pygidial stylus. A morphological character that has been used to distinguish between *O. puerilis puerilis* and *O. puerilis siberti* is the difference in the sclerotization of the mandibles (Paxton & Åkesson, 2007). In hybridization experiments, few of the hybrids between these taxa are viable (Åkesson, 1977). In our molecular analyses, the two subspecies differ from each other by 18% in COI, and based on this difference, they can be considered as two separate species. Basal within the whole *Ophryotrocha-Iphitime* group is *O. longidentata* and *O. globopalpata* (Fig. 5).

Without better basal resolution within *Ophryotrocha* it is difficult to assess the evolutionary origin of the different species in terms of their ecology. However, it is interesting to note that the two most abundant dorvilleid species found on the whale bones, *P. lobifera* and *O. maculata*, are originally described from anthropogenically polluted habitats, in this case beneath fish farms (Oug, 1978) and in the vicinity of pulp mills (Åkesson, 1973) respectively. The worms were feeding off the bacterial mats covering the whale bones in our study. We can assume that these species evolved as specialist on bacterial mats at whale-falls or other natural large food-falls at the seafloor, and have more recently been able to utilise anthropogenic waste as a source of food.

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